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13. ABSTRACT *(Maximum 200 words)*

The overall objectives of AFOSR-sponsored studies in Dr Van Cauter's laboratory are to delineate the synchronizing effects of physical exercise and exposure to darkness on the human circadian system and to test the hypothesis that additive effects of adequately timed exposure to pulses of bright light, darkness and exercise may result in large, immediate phase-shifts of human rhythms. Mr. Buxton performed a series of studies related to the first specific aim of the project, namely, to define the role of exercise intensity and duration in causing phase-shifts. The major rationale for examining the role of exercise intensity and duration in causing phase-shifts is to determine whether exercise seesions that can more readily be achieved in real life conditions than a 3-hour period of arm and leg exercise (which was the exercise period used in our previous studies), will have similar zeitgeber potency. Additionally, the use of a shorter duration, higher intensity stimulus would result in more clear-cut neuroendocrine correlates correlates of exercise and may provide important insights regarding the phase-dependence of exercise-induced neuroendocrine activation.

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Basic Mechanisms and Implications of Non-Photic Entrainment of Circadian Rhythmicity
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Department of Medicine
University of Chicago

YEAR 2 - Technical Report
Sep 1, 1993 - Aug 30, 1994

A. Students supported

1. Mr. Orfeu Buxton, Soc. Sec. # 169-64-9561. Supported on grant from 9/1/93-2/28/94. There were only funds to cover a stipend for Mr. Buxton for part of the 02 year of this project. However, while his stipend came from other sources for the remainder of the year, he continued to work on AASERT supported projects.

2. Mr. Jonathan Wisor, Soc. Sec. # 138-70-1758. Supported on grant from 9/1/93-8/31/94.

B. Research Activities: Mr. Orfeu Buxton.

Mr. Buxton has performed both animal research and human research on the feedback effects of the activity-rest cycle on the circadian clock.

Project title for animal studies: Physiological consequences of disassociating sleep and wake from circadian clock controlled rhythms.

Background:
Major physiological changes take place as humans move in and out of sleep. Many of the physiological systems influenced by the states of sleep and/or wake are also under the regulatory control of the circadian, or 24-hr, clock system which itself normally regulates the timing of the sleep-wake cycle. The circadian control of the sleep-wake cycle insures that the multitude of physiological processes regulated by the clock and the sleep cycle normally receive coordinated, and presumably adaptively significant, temporal signals from the circadian and sleep systems. Since sleep and the 24-hr clock influence many brain controlled bodily functions, any uncoupling of these control systems from one another could have serious health consequences for the organism. Interestingly, humans are essentially the only species in which the mind can override the neural sleep and circadian centers, with the result that periods of sleep and

wakefulness (and the processes regulated by these altered states of consciousness) can occur out-of-phase (i.e. be disassociated) with the circadian clock and the many rhythms under its control. Indeed, this uncoupling of sleep and circadian controlled processes is so unique to humans, that no animal model even exists to study its physiological consequences. Mr. Buxton has developed just such an animal model, and is determining the physiological consequences of a disassociation between two neural centers which regulate 24 hr rhythmicity; a disassociation which can occur in response to cognitive decisions in humans.

Ongoing Experiments:

Ten-week old male and female hamsters maintained on an LD 14:10 light-dark cycle are individually housed in a cage containing a running wheel that allows for the continuous collection via an on-line computer of running wheel activity. After a 2-3 week period of adjustment to the running wheel, three groups of hamsters (10-12 animals per group) areexposed for three weeks to a forced exercise/work regime whereby they must remain active for a 7-hr period of time beginning at either lights on, 7 hrs after lights on, or at lights off. Note that this latter group represents a control group since animals will be forced to be active at a time when they are normally active anyway. A second control group of animals remains untreated during this time period. All four groups are then transferred to DD for at least another month and various rhythms are monitored under free-running conditions.

Because similar experiments have never been performed, it is difficult to predict the results from these studies. However, the proposed studies will enable us to test a number of hypotheses and to address a number of different questions that relate to the relationship between the sleep-wake cycle, the circadian clock system and the various physiological systems regulated by them. Among the hypotheses to be tested are:

1. Disassociation between the entrained circadian clock and the timing of activity will affect the overall temporal organization of the animal and these effects will persist even after the period of forced disassociation is terminated. Of particular interest will be to determine how forced disassociation affects the phase relationship between the activity, temperature and drinking rhythms. These studies will enable us to determine if the effects of activity during the first half of the sleep phase are different than those induced by activity during the second half of the normal sleep phase, and whether there are progressive changes which develop over time during prolonged disassociation between the clock and the timing of activity.

2. Chronic forced activity during half of the normal sleep period will have effects on sleep and activity during the remainder of the sleep period as well as during the normal active period. Again, these studies will enable us to determine if the

effects of activity during the first half of the sleep phase are different than those induced by activity during the second half of the normal sleep phase, and whether there are progressive changes which develop over time during prolonged disassociation between the clock and the timing of activity.

3. Chronic forced activity during half of the normal sleep period will have effects on GH release during the remainder of the sleep period as well as during the normal active period. In hamsters there is a diurnal rhythm in growth hormone release such that about twice as much growth hormone is released during the night or active phase of the animal. Growth hormone is released in pulses of similar frequency (but different amplitude) during the day and night. This is in contrast to humans in which a major portion of GH is released during the sleep phase in association with the first bout of slow wave sleep (SWS). It has been hypothesized that rodents do not show a sleep associated pulse of GH release because they do not have a consolidated long period of wake followed by a single consolidated long period of sleep. In the present study we will be able to determine if a prolonged period of wake leads to a pulse of GH release (possibly in association with a bout of SWS) when the animal is then allowed to sleep during the normal sleep or active phase.

4. Disassociation between the entrained circadian clock and the timing of activity will affect the timing of the circadian rhythms of circulating melatonin and corticosterone levels. Recent studies in humans indicate that exercise during the normal sleep time can induce phase shifts in these rhythms, although it is not known if changes in the timing of the sleep-wake cycle can influence these rhythms in an animal model.

5. Disassociation between the entrained circadian clock and the timing of activity will affect the timing of the circadian endocrine and hormonal events that lead to behavioral estrous and ovulation. It is of particular interest to examine the effects of internal disassociation of activity and the clock on the reproductive system since this system requires the coordination of various temporal programs for normal function, and is critical for the survival of the species.

Project title for human studies: Phase-shifting effects of light and activity on the human circadian clock

Background:

The overall objectives of AFOSR-sponsored studies in Dr. Van Cauter's laboratory are to delineate the synchronizing effects of physical exercise and exposure to darkness on the human circadian system and to test the hypothesis that additive effects of adequately timed exposure to pulses of bright light, darkness and exercise may result in large, immediate phase-shifts of human rhythms. Mr. Buxton performed a series of studies related to the

first specific aim of the project, namely, to define the role of exercise intensity and duration in causing phase-shifts. The major rationale for examining the role of exercise intensity and duration in causing phase-shifts is to determine whether exercise sessions that can more readily be achieved in real life conditions than a 3-hour period of arm and leg exercise (which was the exercise period used in our previous studies), will have similar zeitgeber potency. Additionally, the use of a shorter duration, higher intensity stimulus would result in more clear-cut neuroendocrine correlates of exercise and may provide important insights regarding the phase-dependence of exercise-induced neuroendocrine activation.

Ongoing work

To examine the role of exercise intensity and duration on the magnitude of exercise-induced phase-shifts, seven healthy young men were each studied under three experimental conditions. The three conditions were presented in randomized order and were separated by 2 weeks. The study conditions included: continuous wakefulness in recumbent position, a 3-hour exercise period at moderate intensity (i.e. alternating workload of 40% and 60% of maximum capacity on an arm-and-leg exerciser, as in our previous study) and a 1-hour period of high intensity exercise (including 10-min warm-up, 40 min of stair climbing at 75% of maximal capacity, and a 10-min cool down). In each condition, the stimulus was administered at the same clock time, i.e. centered around 01:00, which is approximately 4 hours before the timing of the minimum of body temperature. The experimental work has been entirely completed. Hormonal assays are in progress. The precise timing of the stimulus wiht respect to the estimated timing of the temperature minimum will be deduced *a posteriori* from the timings of the onsets of the TSH and melatonin rises when the assay procedures will be completed. The phase-shifts observed in response to low and high intensity exercise exposure will be compared to those occurring during the baseline study by analysis of variance for repeated measures.

This timing of exercise exposure was selected for two reasons: 1. in our previous study, we demonstrated robust phase-delays in response to a 3-hour pulse of moderate exercise presented at this circadian time; 2. the exercise period starts late enough (i.e. at 23:30 at the earliest) to allow the observation of unmasked TSH and melatonin rises prior to stimulus exposure.

For the baseline condition and for the condition with the 3-hour exercise pulse, the data obtained using this protocol will be used to perform group comparisons between the six subjects studied at approximately the same circadian time in our previous study (i.e. during constant routine conditions throughout the study) and

those studied with the current design (i.e. with a nocturnal sleep period intervening between stimulus exposure and measurement of endogenous phase position on the next day). While experimental as well as theoretical evidence indicate that the insertion of sleep at the habitual time does not have in itself phase-shifting effects, and would not modify the phase-shifts induced by light exposure, this may not be the case for phase-shifts induced by exercise. This comparison will thus provide important additional information for the design of protocols using late evening exercise exposure to cause phase-delays of human rhythms.

C. Research Activities: Mr. Jonathan Wisor.

Mr. Wisor has primarily been involved in studies over the past year which seek to determine the effects of light on the expression of late response genes in the SCN.

Project title: Light exposure induces VGF mRNA in the hamster suprachiasmatic nucleus.

Background:

The VGF gene was identified in a screen for mRNAs induced by nerve growth factor in PC12 cells. The gene encodes a 90 KDa protein of unknown function that has been localized immunocytochemically to the rodent circadian pacemaker, the suprachiasmatic nucleus (SCN). There is a consensus cAMP responsive element (CRE) beginning 82 base pairs upstream from the transcription start site of the VGF gene. Because the CRE has been linked to the induction of other genes in the SCN by light, we hypothesized that light pulses would induce VGF expression in the SCN.

Ongoing Experiments and Findings:

Male Syrian hamsters aged two to three months were individually caged in constant darkness with continuous access to running wheels for seven circadian cycles. Running wheel activity was monitored for the purpose of determining the circadian period of each animal. At circadian time 19 (seven hours into the active period, or subjective night, of the animal) during the seventh day of continuous darkness, animals were exposed to a five minute pulse of monochromatic light (wavelength = 503 nm, intensity = 100-150 microWatts). Two animals were sacrificed at each of five time points following the light exposure: 1 hr, 2 hr, 3 hr, 4 hr, 6 hr. Six dark control animals were placed in the light pulsing apparatus at circadian time 19 but not exposed to light. Two of these controls were sacrificed at each of three time points after

handling: immediately, 3 hr, 6 hr. In situ hybridization was performed on 20 micron coronal sections of the brains with a radiolabelled VGF antisense riboprobe. The hybridized sections were exposed to nuclear emulsion for approximately three weeks. Developed nuclear emulsion was examined for hybridization signal by dark field microscopy.

No specific VGF hybridization signal was found in the SCN of dark control animals. In the SCN of animals exposed to light and sacrificed 1 or 2 hours later, hybridization signal was only slightly higher than background levels. However, there was a clearly visible increase of hybridization signal at 3, 4 and 6 hours after the light pulse. Thus, light exposure during the subjective night induces VGF mRNA in the SCN of Syrian hamsters. Interestingly, the time course of induction is much slower than that of other known light-inducible genes of the circadian system. VGF induction may therefore be a secondary response rather than an immediate early response to light in the SCN.

In a second series of studies, male hamsters aged 2-3 months were individually housed with continuous access to running wheels. Following one week of entrainment to an LD14:10 cycle the animals were maintained in DD for a period of 7 days. In one experiment, three animals were sacrificed in DD at each of six circadian times (CT2, 6, 10, 14, 18, 22). In a second experiment, two animals were exposed to a five minute light pulse at each of five times (CT3, 9, 14, 19, 21) and sacrificed three hours after that pulse, along with a dark control animal. In situ hybridization was performed on coronal sections of the brains with a radiolabelled *vgf* antisense riboprobe. The results of the first experiment indicated that there is a circadian rhythm of *vgf* mRNA in the SCN in DD conditions. The level of mRNA peaks in the late subjective day and reaches its nadir in the late subjective night. The results of the second experiment indicated that *vgf* is induced at the mRNA level by a light pulse in the subjective night, a time when a light pulse also induces a behavioral phase shift. Interestingly, the induction of *vgf* does not appear to be an immediate early response, as it is detectable for at least 3-6 hrs following a light stimulus. Furthermore, *vgf* induction by light is blocked by anisomycin, a protein synthesis inhibitor. Thus, *vgf* appears to be a late response gene that is regulated both by the circadian clock and by light.